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BOTANICAL GAZETTE

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THE STAMINATE CONE AND MALE GAMETOPHYTE OF PODOCARPUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 114

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(WITH NINE FIGURES AND PLATES VIII AND IX)

Knowledge of the gametophytes of the Podocarpineae is at the present time limited to that contained in three brief papers. In 1902 COKER (3) published an account of two species of Podocarpus; in June 1907 JEFFREY and CHRYSLER (5) added some new facts concerning Podocarpus and Dacrydium; and in September of the same year Miss YOUNG (14) gave an excellent résumé of the subject of the male gametophyte and added several new and interestesting details for Dacrydium. COKER records that in *Podocarpus coriacea* there are produced two primary prothallial cells, both of which may divide amitotically; a tube nucleus; and a primary spermatogenous cell, which gives rise to a stalk cell and a body cell. The body cell undergoes nuclear division and one of the nuclei is then extruded from the cytoplasm, leaving a single functional male cell. JEFFREY and CHRYSLER add to this the interesting details that from the two primary prothallial cells there may arise by subsequent division as many as eight prothallial cells. Of still greater interest is the record of the "proliferation" of the primary spermatogenous cell, whereby there arise three cells placed transversely, the middle one of which is considerably larger than either of the others. Miss YOUNG reports that in Dacrydium one or both of the primary prothallial cells may divide once. From the primary spermatogenous cell arise two cells, one of which is usually sterile, the other of which functions as the body cell. The interesting observation is made that there is a tendency for both derivatives of the primary spermatogenous cell to function as body cells.

The material for the present study was secured through the kindness of Dr. L. COCKAYNE of Christchurch, New Zealand, and Miss INEZ FRANCES STEBBINS of the Huguenot College at Wellington, Cape Colony, South Africa. To both are due our thanks for their kindness in obtaining the material at no inconsiderable personal inconvenience. Though the collections were necessarily few, yet they include a considerable range of developmental stages in each of the species. The material was fixed in a 5 per cent. solution of commercial formalin in 70 per cent. alcohol. The fixation has proved unexpectedly good, except for stages from the rounding-up of the mother cells to the time when the microspores have a good firm wall and abundant contents. The shrinkage and general distortion within the limits just mentioned have rendered necessary the omission of a number of what appear to be rather interesting and perhaps important details. Some of these will be mentioned briefly in the general account. It is hoped that other material so killed and fixed as to show details of cytological structure will be in hand within the near future. The preparations have been imbedded in 54° paraffin, cut 3–5 μ, and stained in Haidenhain's iron alum hematoxylin stain, alone or counterstained with various aqueous stains such as orange G, Bismarck brown, erythrosin, etc., and with the saffranin gentian-violet combination. Three species have been examined, though not covering exactly the same ground.

Observations

The cones of *P. totarra Hallii* are of a generally oval shape, varying from 3 or 4 to 15 or 20^{mm} in length, and from 2 to 4^{mm} in diameter. They are usually in pairs, one being large and one small. The base of the cones is surrounded by several closely appressed, rigid, scalelike bracts. The peduncle is quite short or almost lacking. The cones of *P. nivalis* are somewhat shorter, not paired, and decidedly slenderer. The cones of the third species (the label of which was lost in transit) are similar in shape to the first ones, but are smaller. In other respects the cones are very similar. *P. totarra* is one of the tallest of forest trees and *P. nivalis* a very small shrub, sometimes creeping. The third species may be *P. elongata*, a common forest tree of the South African region. This is a mere inference from its abundance in the region from which this material

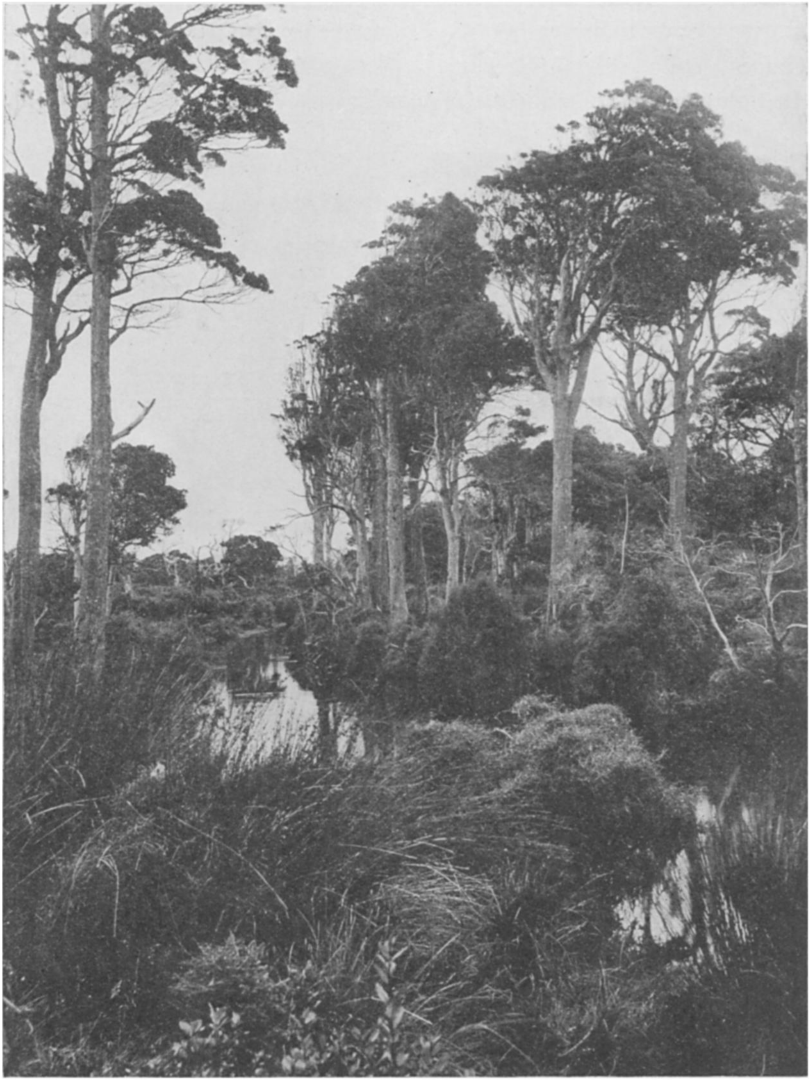

FIG. A.—Group of *P. dacrydioides* in remnant of taxad forest, N. Z.—Photograph by J. CROSBY SMITH.

was received, and from the fact that I have received other material from the same source belonging to this species.[1]

In cross-section a staminate cone shows about ten to twelve sporophylls, each with two sporangial cavities (*fig. 1*). The vascular system seems to be rather weakly developed, but shows a distinct endarch collateral bundle (*fig. 3*) corresponding to each sporophyll. Just outside of the bundle there usually is found a resin canal (*fig. 1*).

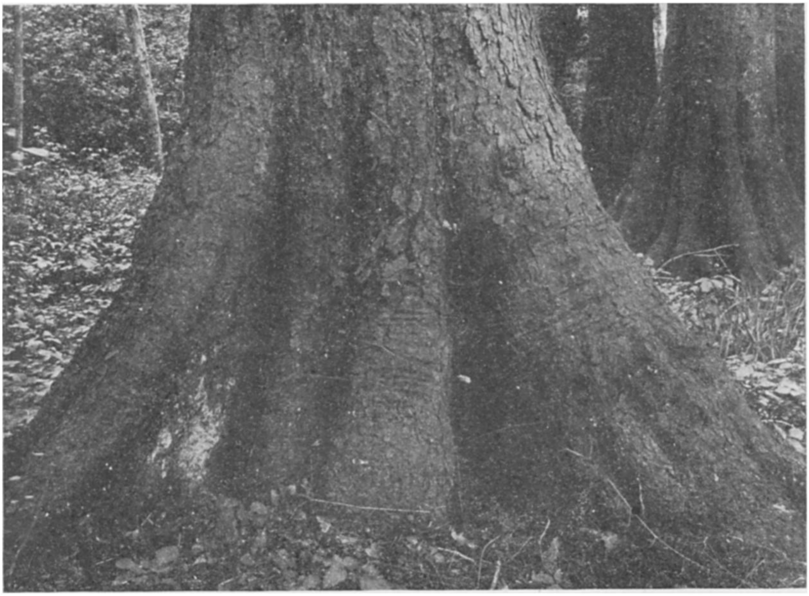

FIG. B.—Buttressed base of *P. dacrydioides*, in ancient forest of Canterbury Plain, N. Z.—Photograph by L. COCKAYNE.

The resin canals contain little material of any sort in the preparations available for study. The distinctly glandular cells lining them (*fig. 2*) suggest, however, that they are functional. The number varies in different strobili, but in those examined did not fall below six nor exceed eighteen or twenty.

The sporophylls as seen from the upper surface are somewhat spatulate, with a rather pointed apex. *Fig. 4* presents an outline

[1] We have received from Dr. COCKAYNE two excellent photographs of *P. dacrydioides*, which are reproduced on account of their general interest (*figs. A* and *B*).

of a longitudinal section somewhat to one side of the middle, and will give an idea of the side view, as well as the relation of the sporangial cavity to the sterile portion of the leaf. *Fig. 6* shows a cross-section of a sporophyll near the middle of the sporangium. The sporangia are somewhat less prominently exposed on the under side than those of Pinus, for example. A single weakly defined vascular bundle (*fig. 6*) traverses the upper part of the central sterile septum and is sometimes accompanied by a resin canal lying below it and between the sporangia. The wall of the sporangium varies in thickness somewhat, but in general on the freely exposed part it is about four or five cells thick (*fig. 5*). One or two of the inner layers are slightly differentiated as a tapetum. This is not very evident and does not occur until the sporogenous tissue has nearly reached the mother cell stage. The tapetum does not long persist, but disappears as a functional tissue about the time the young spores have formed a thick wall and wings. All of the wall proper, except the outermost layer of thickened cells, may break down before the spores are shed.

The earliest stages of the sporogenous tissue observed (in *P.* sp.) lacked one or two divisions of the mother cell stage. *Fig. 7* will serve as a typical representative cell of the sporogenous tissue at this stage. The walls are thin and delicate, as is usually the case, and the cytoplasm stains somewhat more densely than that of the wall cells. It was possible to secure very good preparations of this, considering the nature of the killing and fixing agent. The cells were slightly shrunken, so as to have pulled away in many places from one another or from their own walls. *Fig. 7* will show the principal facts. The cytoplasm does not seem to present either a typically reticulated or a foam structure, but rather resembles a sort of flocculent precipitate scattered more or less irregularly through the cell. These flocculent masses may assume a sort of feathery, filamentous, more or less branched form, or they may appear as scattered masses of irregular shape. The filaments and other masses do not seem to be connected in any definite manner, but to be distributed through the cell by chance. No stored food can be detected microscopically in the cytoplasm at this time, though the parenchymatous cells of the axial portion of the strobilus contain considerable quantities of large starch grains. The wall cells of the sporangium do not at this time, as in some sporangia (Ophioglossum, for example), contain starch, but are relatively poor in cytoplasm and cytoplasmic inclusions. There is relatively little change in the cytoplasm of the sporogenous tissue as growth advances, except that it grows gradually less dense until at the time of the formation of the young spores there is almost no stainable substance left.

The nuclei of the sporogenous cells (*fig. 7*) previous to the mother cell stage show a structure that is very like that of the cytoplasm just described, except that it is denser and there is more of a tendency to aggregate itself into disconnected masses. There are one or more large nucleoli present in the nucleus at this stage, as there seem to be in the resting nuclei of all the stages of all the tissues examined.

As the nucleus approaches division, the nuclear substance begins gradually to arrange itself into filaments or strands (*fig. 8*) which may end freely or anastomose with other filaments or masses. About the same time the hitherto nearly uniformly staining material begins to stain differentially, so that filaments seem to link together knots of more deeply staining material. Whether this indicates a difference of substance, as some think, or merely a difference in the state of aggregation, as has been recently maintained by several authors, cannot be said with certainty. However, the gradual derivation of this structure from the preceding one, in which everything seems homogeneous, would certainly point to the latter interpretation as the correct one. During this time the nucleolus (or nucleoli) gradually loses its density, as shown by the staining reactions. It continues to do so until it finally does not stain at all or has disappeared entirely. *Fig. 9* shows a surface view of a nucleus in which the filaments have become more regular and somewhat thicker. In *fig. 10* is shown a spirem, into which the filamentous stage gradually passes, in which the deeply staining knots on the spirem are unusually prominent. The figure shows nearly all of the spirem that was included in a 3-μ section; therefore it probably contains one-third to one-half of the entire spirem, since the nucleus is about $7 \times 10\,\mu$. In some cases the spirem consists of darkly stained masses connected by much smaller threads, and in others, as shown in *fig. 10*, by broader strands which seem to be of different material because of the considerable difference in depth of staining.

The spirem gradually becomes denser and ramifies all through the nucleus (*fig. 11*), the nucleolus disappears, and finally the spirem breaks into elongated loops, which arrange themselves into a tangled mass at the equatorial plate. The exact method of division was not made out owing to the close tangling of the long chromosomes. In the anaphase the chromosomes appear as twenty-four straight elongated rods of about half the length of those entering the mitosis (*fig. 12*).

As the chromosomes approach the poles, the polar ends draw together and the others spread out, so that the daughter nucleus usually has a concavity on the polar side. The chromosomes gradually lose their density and put out processes on one side or the other to fuse with those put out by neighboring ones, thus establishing a sort of reticulate structure (*fig. 13*). This gradually passes back into the stage shown in *fig. 7*. In the reorganizing nucleus one large nucleolus may appear or several small ones, but I did not attempt to discover the origin of them or their relation to one another.

When the cells have reached the mother cell stage, they break loose from one another and round up in the usual manner. As already remarked, the mother cells killed and fixed very poorly, so that only a very meager and perhaps uncertain account of them can be given. About the time the mother cells round up many of them begin to abort; this abortion seemingly may occur as late as the young spores. Up to this time and on to the time of the divisions in the young microspores the development of the sporogenous tissue seems to be nearly simultaneous.

After the mother cells have rounded up, the nucleus soon passes into a condition in which its contents stain so nearly uniformly that it has not proved possible to disentangle its separate constituents. If stained deeply, it appears absolutely uniform, and if the stain be then slowly and gradually drawn, the homogeneous appearance gives way to a sort of pebbled one. Sometimes it looks as if this might be due to the parts of a large and long spirem being crowded closely together. This interpretation is strengthened by the fact that in some cases one can see what looks like the end of a loop pushing out the nuclear membrane. In other cases the appearance is more that of a great many lumps of some plastic substance thrown into a tight mass. Whether these appearances are normal or not I cannot say. That they represent the only appearances that the present material shows is certain. In the face of the numerous and fairly uniform accounts of sporogenesis in which no such phenomena have been recorded, it is certainly entirely proper at present to ascribe these unusual phenomena to the effects of the reagents. Nothing any more nearly resembling synapsis was seen. It should be noted that of some hundred strobili cut only five showed cells in this stage. However, three of these strobili are the only ones that have shown the reduction divisions. A few sporangia had cells like those shown in *fig. 14*, where the chromosomes are beginning to appear, being almost completely concealed in a mass of granular matter. In the figure the difference in intensity of stain between the chromosomes and the imbedding substance has been greatly exaggerated for the sake of clearness. One gets two views of these developing chromosomes, one in which the chromosomes are mostly seen in cross-section and one in which they lie lengthwise. In some fashion (which the density and abundance of the imbedding substance prevent being made out clearly) out of this comes a rather sharply pointed spindle at whose equator are arranged the short apparently bivalent chromosomes (*fig. 15*). Some preparations seemed to favor the idea that these apparently bivalent chromosomes then split longitudinally. After passing to the poles (*fig. 16*) they became surrounded by a nuclear membrane, but did not, in the few preparations of this stage observed, lose their identity in a resting nucleus. A very few cases of the homotypic mitosis are shown in the preparations and all of these are in the telophase, as shown in *fig. 17*. Walls are not formed till after second division.

Beyond the telophase of the second division the preparations show nothing until after the formation of the wall around the young spores. These are so badly shrunken and distorted that no attempt has been made to figure them. It is observable, however, that the nucleus is at first rather small and oval and lies to one side of the spore cavity, most of which is occupied by an enormous vacuole. *Fig. 18* shows a microspore after the contents have become more abundant. At this time starch may or may not be present; later it becomes abundant and occurs in relatively large grains (*figs. 30, 31*). The wall has a thick exine much roughened on the outer surface and a distinct intine. The spore coat is usually much thinner on the side away from the point where the prothallial cells will later lie. The external appearance of the pollen grain bears a considerable resemblance to that of Pinus, except that it is smaller, rarely reaching more than 35 μ in the greatest diameter (exclusive of the wings).

The microspore nucleus is large (*fig. 18*) and contains apparently two sorts of material. There is a more deeply staining substance distributed in irregular masses through a very fine ground substance. As the nucleus prepares for division, the amount and density of the more deeply staining substance increases in amount, assumes a more definitely reticulated structure, and finally passes into a rather thick and comparatively short spirem (*fig. 29*). This spirem breaks up into the chromosomes (*fig. 28*), which often still show the distinct segmentation into lighter and darker segments that has already been referred to in discussing the sporogenous tissue. This segmentation is not always found in the chromosomes or even in the spirem. It might be supposed that the different appearance is due to differences in the depth of stain, but this does not seem to be true. The chromosomes are rather long and considerably twisted at the metaphase, but in dividing and passing to the poles they apparently shorten up (*fig. 31*). What has been said of the first division applies equally well to the next, so far as the few mitoses observed show.

One or two prothallial cells are cut off and then the antheridial initial divides to produce the tube nucleus and the primary spermatogenous cell (*fig. 32*). In *P. nivalis* two primary prothallial cells, which do not subsequently divide, seem to be the rule, though one example of the division of the first prothallial was observed. The pollen in the same sporangium varies in the stage of development from microspores to the stage shown in *fig. 32*. These sporangia were, in some cases at least, shedding their spores.

In *P. totarra Hallii* one primary prothallial cell may be cut off, after which the free nucleus divides to form the primary spermatogenous cell and the tube nucleus (*fig. 21*); or two primary prothallial cells may be cut off before the tube nucleus is separated from the primary spermatogenous cell (*figs. 20, 22*). When only a single primary prothallial cell is cut off, it may (*fig. 19*) or may not (*fig. 21*) divide again. Far more frequently it does divide anticlinally. No case in either species has been observed where either prothallial cell divides periclinally. In case two prothallial cells are cut off, both usually divide anticlinally (*figs. 23, 26, 27*), although the division may be long delayed or fail wholly (*text fig. 4*). The first one may remain undivided, or in rare cases may divide twice, so that the first tier of prothallial

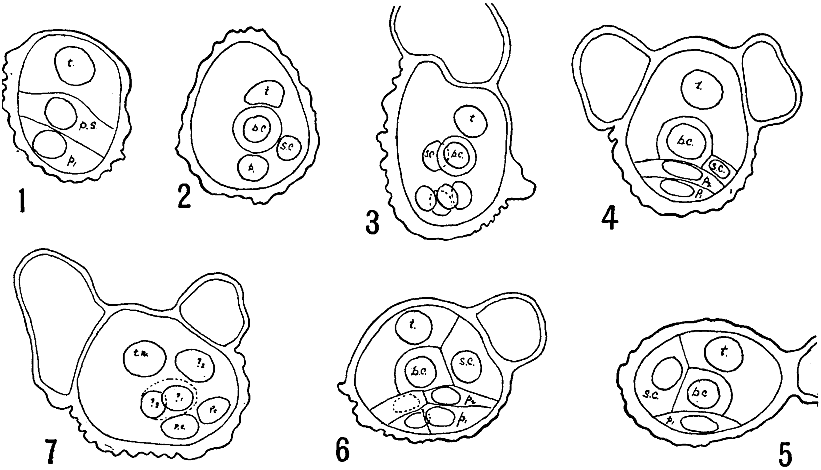

FIG. 1. Transverse section of a male gametophyte consisting of one prothallial cell (p_1), the primary spermatogenous cell (ps), and the tube nucleus (t).—FIG. 2. Male gametophyte in which the primary spermatogenous cell has divided into the body cell (bc) and the stalk cell (sc), and in which all the gametophytic walls have broken down.—FIG. 3. Same as the preceding except that there are four prothallial nuclei instead of one; it is uncertain whether they have been derived from one or two primary prothallial cells.—FIG. 4. A gametophyte with two primary prothallial cells (p_1, p_2) which have not divided, though the pollen is presumably ready for shedding.—FIG. 5. A gametophyte with a large stalk cell placed transversely to the axis of the body cell.—FIG. 6. Same as preceding except that there are four derivatives of the primary prothallial cell.—FIG. 7. A gametophyte in which the origin of the free nuclei is not easily traced; see text for discussion.

cells contains four cells. The second one almost always divides once and usually twice. Thus there may be one, three, four, six, or eight prothallial cells; six is much the commonest number, and one or eight the rarest. In case only one prothallial cell is cut off, its nucleus remains large and the cell itself is often much larger than when two are cut off (*text fig. 1*). Sometimes the three resulting nuclei then lie in a row across the spore (*text fig. 1*) and differ but little from one another in appearance. The walls separating them then run nearly straight across, and it is only by courtesy that the tube nucleus can be said to be free in the spore cytoplasm. To all intents and purposes it is as much walled-off into a cell of its own as the prothallial nucleus, for both eventually are freed in a common cytoplasm (*text fig. 2*). Further, it is to be noted that in this case the prothallial cell does not have a fixed position with respect to the wings, as in the ordinary course of events.

After the tube nucleus has been cut off, the primary spermatogenous cell divides transversely, giving rise to two cells (*fig. 25*). Usually these cells differ markedly in size, the smaller lying to one side and the larger lying nearly in the center of the pollen grain (*fig. 26*). In this case one may safely use the usual terms for them and speak of the larger as the body cell and the smaller as the stalk cell. It not infrequently happens that the two derivatives of the generative cell are about equal in size (*figs. 24, 27; text figs. 5, 6*), though even then one is centrally placed and the other laterally. Before the division of the generative cell, the second primary prothallial cell has usually divided at least once. In this case the generative cell often sinks down among its derivatives (*figs. 23, 27*). The stalk cell is sometimes placed transversely to the body cell (*text figs. 5, 6*). Whether these large stalk cells will produce male cells or not can only be conjectured, though the fact that some of them seem to retain their cytoplasmic envelope, almost as distinct from the cytoplasm as the body cell itself, up to a certain early stage, would lead one to suppose that in some cases they might do so. *Text fig. 7* may show three derivatives of the generative cell and two prothallial cells in addition to the tube nucleus. One of the three derivatives of the generative cell has no cytoplasmic sheath and is probably a stalk nucleus; the two lying close together are ensheathed, though I have been unable to ascertain definitely whether each has a distinct sheath or whether both lie in a common sheath, or whether perhaps one of them is not merely beneath the cytoplasmic sheath of the other. If either the first or second supposition is true, there still remains the question whether the two cells are body cells or male cells. If the third supposition is true then there are four prothallial nuclei, a generative cell, and the tube nucleus.

In a number of preparations there seemed to be a cell on the side of the body cell opposite the stalk cell (*fig. 27*), but in no case could I satisfy myself of the presence of a nucleus; though in view of the figures published by JEFFREY and CHRYSLER it was diligently looked for. In *fig. 27* is shown a mass at one end of this "cell," which may stand for a degenerating nucleus, although I think not. It is very indistinct and may very well be a mere aggregation of slightly denser cytoplasm. By constructing a model of the cell complex, where the body cell has sunk down between the cells of the second tier of the prothallus, and then cutting it so that the section will pass about centrally through stalk and body cell and include most of one-half of them and take a slice off the prothallial cells lying beyond them, it is possible to get preparations which show cell walls in the position shown in the figure. Hence it is not certain that in this species there are two lateral derivatives of the generative cell, as seems to be the case in the species mentioned by JEFFREY and CHRYSLER (**4**).

The dividing nuclei of the species of Podocarpus here investigated show uniformly twelve chromosomes in cases where they could be certainly counted (*figs. 14, 27, 32*), and twenty-four were counted several times, with less certainty, in the sporogenous tissue of *P.* sp. In this it conforms to the count for all other gymnosperms so far as known except Taxus, with eight and sixteen (STRASBURGER **12**), and Sequoia, with sixteen and thirty-two (LAWSON **7**).

It is not possible to speak with certainty concerning the stage at which the pollen is shed without knowing over how long a time shedding continues, and without actually having gathered pollen as it is shed naturally. But from cones that were apparently ready to shed their pollen, stages were obtained in *P. nivalis* running from the microspore to gametophytes with two prothallial cells, generative cell, and tube nucleus. It often happens that both extremes may be found in the same sporangium. In *P.* sp. no cones were apparently old enough to shed their pollen, though the oldest had essentially the same structure as those of the species just mentioned. In the cones of *P. totarra Hallii* the range appears to be still wider, for the oldest stages shown in the plates (*figs. 26, 27; text fig. 7*) occurred along with the youngest. In some cases the cell walls were still intact, while in other pollen grains not more advanced, the nuclei and body cell were free in the common cytoplasm (*text figs. 1, 3, 7*).

Discussion

The facts presented in the preceding account are in the main confirmatory of those already reported by COKER and by JEFFREY and CHRYSLER for other species of Podocarpus; hence their main value is that they offer additional evidence that the phenomena described by these authors are not limited to the species studied by them. These authors and others as well have called attention to the resemblances to the Abietineae in the winged pollen, thick sporangium wall, and general similarity of the staminate strobilus.

The main interest centers about the development of the male gametophyte. COKER (**2**) reported two primary prothallial cells in *P. coriacea*, which divide amitotically. JEFFREY and CHRYSLER (**5**) have recorded as many as eight in the species studied by them. Miss YOUNG (**14**) has also recorded as many as four in species of Dacrydium. THOMPSON (**13**) has found 30 to 40 prothallial cells in Agathis; and LOPRIORE (**8**) has reported a cell complex in Araucaria that reaches about 15 in number before the walls break down, when the freed nuclei may then further divide until as many as 44 may be found in the pollen tube. He interprets these as spermatogenous cells and cites the case of Cupressus as analogous. CHAMBERLAIN (**2**) has already called attention to the fact that the drawings seem to indicate that these nuclei are really prothallial and that there is only one body cell. In this view Miss YOUNG (**14**) has concurred, as it would seem most students of gymnosperms must. RENAULT (**10, 11**) and OLIVER (**9**) have described the multicellular pollen grains of the Cordaitales and Stephanospermum, where as many as 20 cells are found in the male gametophyte; whether they are prothallial or spermatogenous is uncertain.

CALDWELL (**1**) has reported as many as nine or ten body cells in Microcycas, along with a single stalk and single prothallial cell. CHAMBERLAIN in a forthcoming paper reports that Ceratozamia occasionally has four sperms. JUEL (**6**) found a variable number of sperm cells (it is not clear whether he means body cells or male cells) in *Cupressus goweniana.*

It thus seems to be an established fact that more than two prothallial cells and more than two sperms occur in widely unrelated gymnosperms. Though we may all be willing, perhaps, to accept this statement of facts, there is no such unanimity of opinion as to the interpretation of them. It is obvious that one may adopt either of two views: (1) One or two prothallial cells were found in the primitive gymnosperms and in some cases these have divided to form a complex. This would be JEFFREY'S (**5**) "coenogenetic proliferation." In support of this view he has urged that a multiplication of prothallial cells is correlated with "protosiphonogamic" fertilization in the Araucarineae, "since the length of the pollen tube, in the absence of any special conductive tissue, such as is found in angiosperms, calls for a greater development of prothallial tissue." This line of reasoning could hardly be extended to Podocarpus or to any other case now known. If one could find some physical cause applicable to all cases, he might believe that this multiplication of cells is a thing of recent origin in each case. Furthermore, the idea that a multiplication of cells would be forthcoming just when needed is more teleological than accords with modern physiological teaching. If we do not accept this explanation, as I think we cannot, we may turn to the other possibility. (2) Primitive gymnosperms had a multicellular prothallial tissue, and not unlikely a spermatogenous complex of several cells. The evidence for this view is of two sorts, historical and theoretical. Historically we know that the pollen grains of certain Cordaitales (**10**) and Cycadofilicales (**9, 10, 11**) had a considerable tissue of some sort. Whether it was spermatogenous or prothallial is not very material, since this view assumes that both sorts existed somewhere in the ancestry of living gymnosperms and that by a gradual reduction each sort has been reduced to one or two cells. It is of interest to note that all the heterosporous pteridophytes show the same tendency, each of the living genera having a single prothallial cell and in some cases producing as few as four sperms. This assumption makes it easy to account for the facts already related. We have but to suppose that in these cases the forms showing these peculiarities have merely retained their primitive characters or, in cases where they occur only occasionally, that it is a reversion to ancestral conditions. JUEL (6) has already suggested this explanation for the case of Cupressus, and has even gone so far as to suggest a possible sequence in the reduction. At this point one must be on guard against supposing that types which illustrate this reduction series necessarily, or even probably, stand in the same series phylogenetically. It seems to the writer that if this fundamental principle be firmly grasped, it is entirely unnecessary to suppose that because Podocarpus and Pinus are clearly related and because Pinus is older historically, therefore the multiplication of prothallial cells in Podocarpus must be coenogenetic. One need but assume that both are derived from a common ancestral stock and that one has retained the prothallial complex and the other has lost it. Of course if one believes that Podocarpineae have come directly out of living Abietineae, this explanation would not hold. While it is true that the latter are known as far back as the Carboniferous (4) and the former are not known to be nearly so old, it is equally undeniable that we know but little of the plant remains of those parts of the world in which their remains would be most likely to be found.

Summary

1. There are in the species of Podocarpus studied two prothallial cells which may or may not divide. There may be as many as eight prothallial cells in two tiers derived from the two primary ones.
2. Division in the prothallial tissue is mitotic and the prothallial cells do not degenerate.
3. There is a stalk cell and a body cell, sometimes differing very little from one another in appearance; whether both may produce male cells is yet uncertain.
4. The number of chromosomes is twelve and twenty-four.
5. There may be a variable number of cells or free nuclei in the pollen grain at the time it is shed.

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LITERATURE CITED

1. CALDWELL, OTIS W., *Microcycas calocoma.* BOT. GAZETTE **44**:118–141. *pls. 10–13. figs. 14.* 1907.
2. CHAMBERLAIN, C. J. (Review). BOT. GAZETTE **40**:391. 1905.
3. COKER, W. C., Notes on the gametophytes and embryo of Podocarpus. BOT. GAZETTE **33**:89–107. *pls. 5–7.* 1902.
4. JEFFREY, E. C., and CHRYSLER, M. A., On Cretaceous Pityoxyla. BOT. GAZETTE **42**:1–15. *pls. 1, 2.* 1906.
5. ———, The microgametophyte of the Podocarpineae. Amer. Nat. **41**:355–364. *figs. 5.* 1907.
6. JUEL, H. O., Ueber den Pollenschlauch von Cupressus. Flora **93**:56–62. *pl. 3.* 1904.
7. LAWSON, A. A., The gametophytes, archegonia, fertilization, and embryo of *Sequoia sempervirens.* Annals of Botany **18**:128. *pls. 1–4.* 1904.
8. LOPRIORE, G., Ueber die Vielkernigkeit der Pollenkörner und Pollenschläuche von *Araucaria Bidwillii.* Ber. Deutsch. Bot. Gesells. **23**:335–346. *pl. 15.* 1905.
9. OLIVER, F. W., On the structure and affinities of Stephanospermum. Trans. Linn. Soc. London II. **6**:361–400. *pls. 41–44.* 1904.
10. RENAULT, B., Structure comparé de quelques tiges de la flora carbonifère. 1879.
11. ———, Cours de Bot. Foss. **4**:1880–1884. Paris.
12. STRASBURGER, E., Anlage des Embryosackes und Prothalliumbildung bei der Eibe. Festschrift für Haeckel 1–16. *pls. 1, 2.* Jena. 1904
13. THOMPSON, R. B., Preliminary note on the Araucarineae. Science N. S. 22:85. 1905.
14. YOUNG, Miss M. S., The male gametophyte of Dacrydium. BOT. GAZETTE **44**:189–196. *pl. 19.* 1907.

EXPLANATION OF PLATES VIII AND IX

The drawings were made with a Bausch and Lomb camera lucida and Zeiss apochromatic lenses and compensating oculars. The plates have been reduced one-half in reproduction.

PLATE VIII

(*Figs. 1, 2, 5, 7–17* are *P.* sp.; *figs. 3, 4, 6* are *P. totarra Hallii*)

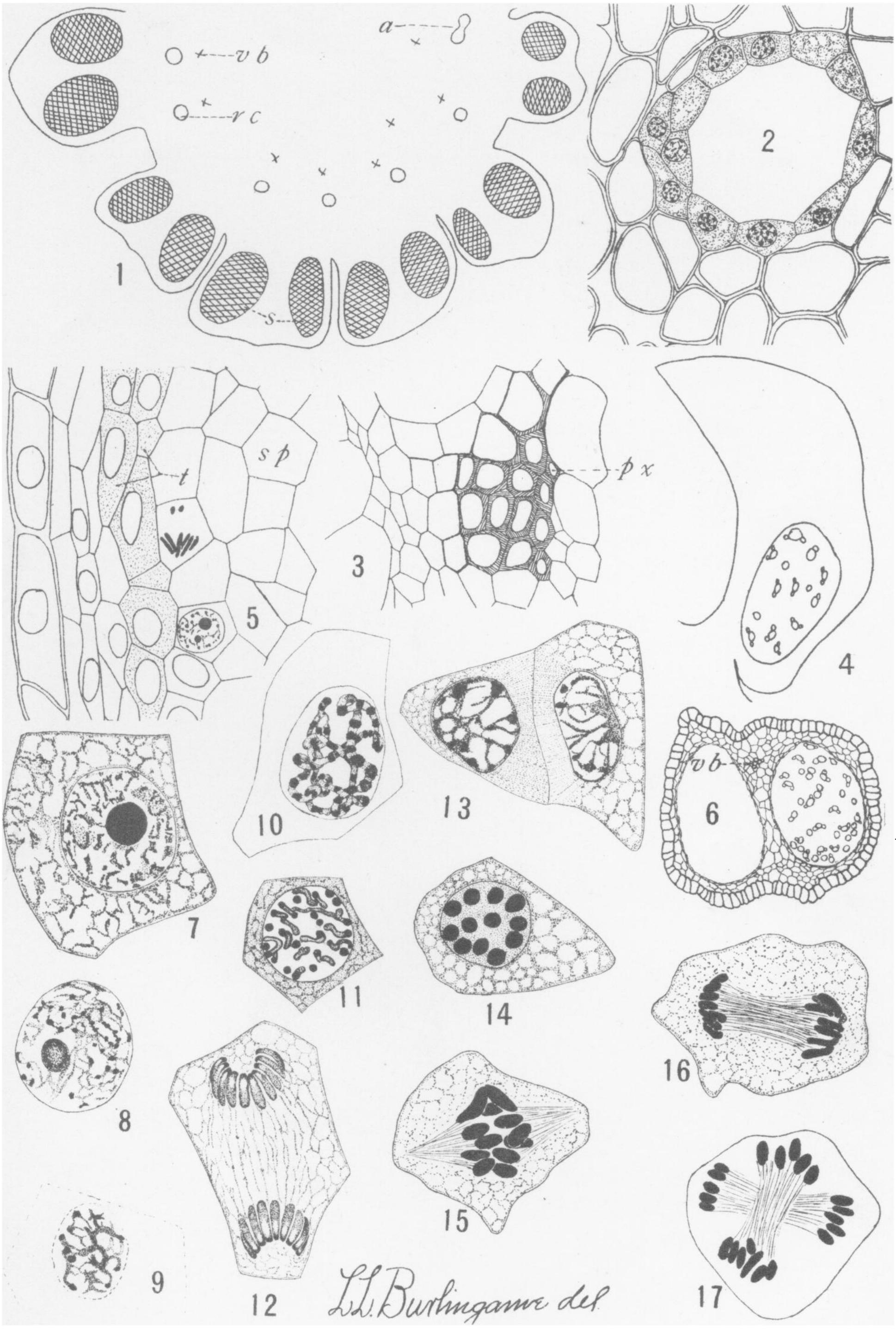
FIG. 1.—Cross-section through the middle region of a staminate cone, showing the relative position of sporophylls and sporangia (*s*), vascular bundles (*vb*), and resin canals (*rc*); the canals sometimes branch as shown at *a*.

FIG. 2.—Cross-section of resin canal.

FIG. 3.—Cross-section of vascular bundle from strobilus; *px* shows position of protoxylem.

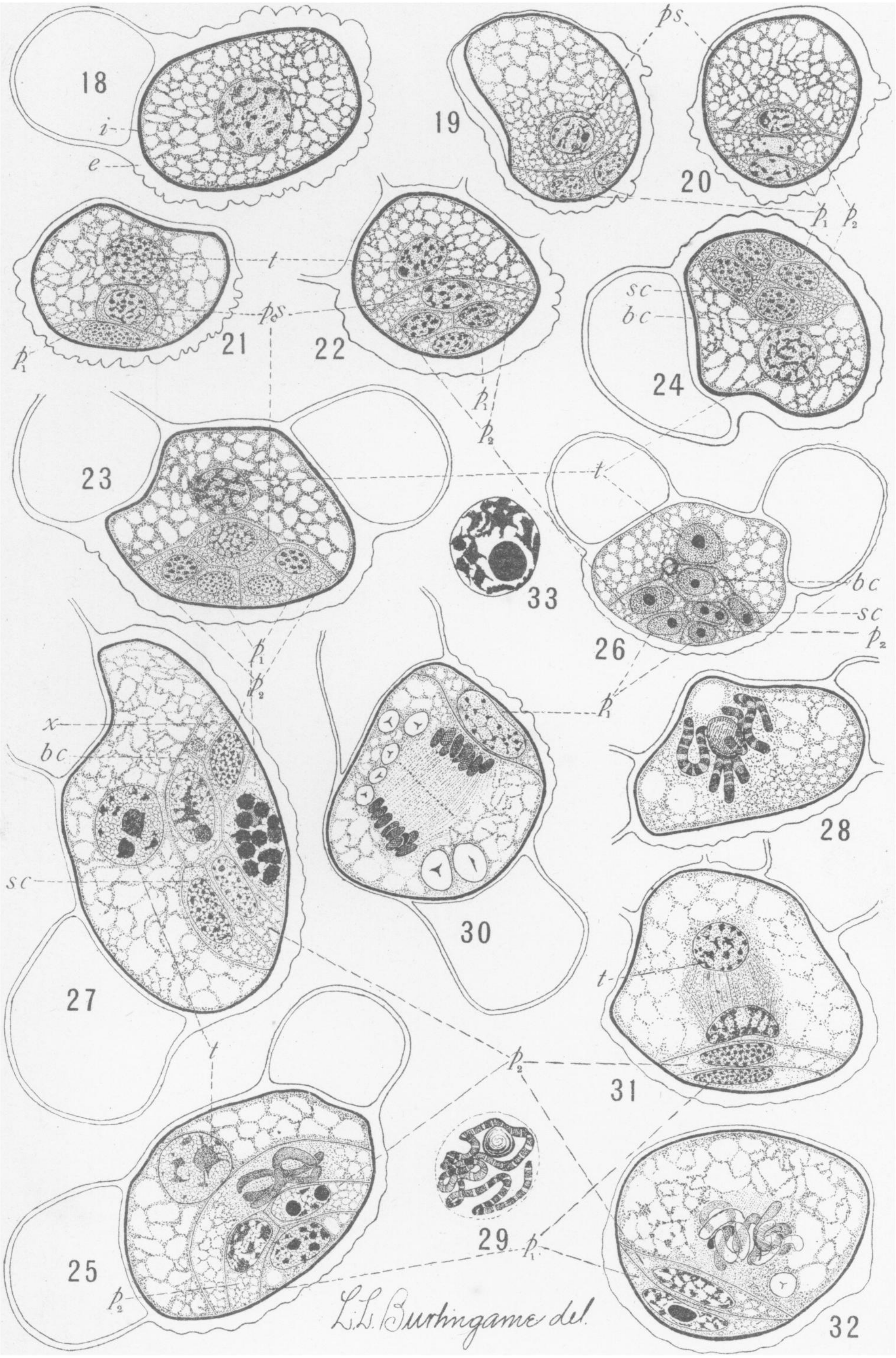
FIG. 4.—Longitudinal section of a sporophyll, and one sporangium of a young cone about 3^{mm} in length.

PLATE VIII



L.L. Burlingame del.

BURLINGAME on PODOCARPUS



BURLINGAME on PODOCARPUS

FIG. 5.—Detail of sporangium of same age as preceding, showing relations of wall, tapetum (*t*), and sporogenous tissue (*sp*).

FIG. 6.—Cross-section through sporangia; *vb*, vascular bundle.

FIG. 7.—Cell of the sporogenous tissue about one division short of the mother cell stage.

FIG. 6.—Detail of a nucleus a little later than preceding, showing the nuclear materials beginning to arrange themselves into threads with here and there deeply staining chromatin knots.

FIG. 9.—Surface view of a still older nucleus showing the strands more distinct and larger.

FIG. 10.—The definitely organized spirem with unusually distinct chromatic knots; the spirem is usually more homogeneous and stains more evenly.

FIG. 11.—A very thin section of a nucleus whose spirem is beginning to segment into chromosomes; compared with *fig. 7*, it shows the variation in size and shape of the sporogenous cells.

FIG. 12.—Longitudinal section of a cell in telophase showing about half of each group of chromosomes; the spindle is curved with the concavity toward the observer.

FIG. 13.—Showing the reconstruction of the daughter nuclei.

FIG. 14.—Mother cell showing the emergence of the chromosomes from the granular mass out of which the spindle arises; the granular materials are actually much denser than shown in this figure and nearly conceal the chromosomes.

FIG. 15.—Longitudinal section of the heterotypic spindle.

FIG. 16.—Late anaphase of the heterotypic mitosis.

FIG. 17.—The homotypic mitosis.

PLATE IX

(*Figs. 18–27, 33* are *P. totarra Hallii; figs. 28–32* are *P. nivalis*)

FIG. 18.—Section of microspore slightly oblique to the wings; *i*, intine; *e*, exine.

FIG. 19.—Male gametophyte showing first prothallial cell (p_1) divided, no second prothallial (p_2) cell, primary spermatogenous cell (*ps*), and in next section the tube nucleus (*t*).

FIG. 20.—Same stage as preceding except that there are here two primary prothallial cells (p_2), the second of which has divided (only one, p_2, of the segments shown).

FIG. 21.—Same as *fig. 19* except that the single primary prothallial cell (p_1) has not divided; *t*, tube nucleus.

FIG. 22.—Male gametophyte showing first prothallial (p_1) undivided, second $(p)_2$ divided once, primary spermatogenous cell (*ps*), and tube nucleus (*t*).

FIG. 23.—Same as preceding except that first prothallial cell (*p*) has also divided and the primary spermatogenous cell (*ps*) has sunk down between the derivatives of the second prothallial cell (p_2); adjacent sections show that there are here four derivatives of the second prothallial cell.

FIG. 24.—Showing the stalk (*sc*) and body (*bc*) cells as well as prothallial cells (p_1, p_2) and tube nucleus (*t*).

FIG. 25.—The mitosis giving rise to stalk and body cells from the primary spermatogenous cell.

FIG. 26.—A usual type of mature gametophyte, having four derivatives of the first primary prothallial cell (p_1), four of the second (p_2), stalk (*sc*), and body (*bc*) cells, and tube nucleus (*t*); nine cells also usual (two p_1, four p_2, stalk, body, tube).

FIG. 27.—Showing the prophase of the division of the first prothallial cell (p_1) and what appears to be a "cell" (*x*) on the side of the body cell opposite to the stalk cell (*sc*); no nucleus could be discovered in this "cell;" see text for further details.

FIG. 28.—Division of the microspore nucleus in *P. nivalis*; a peculiar segmented appearance of the spirem, which has about completed segmentation into chromosomes, is shown.

FIG. 29.—A similar but younger spirem which has not yet begun to segment as the preceding one has done.

FIG. 30.—Anaphase of the division resulting in second prothallial cell (p_2) and antheridium initial.

FIG. 31.—Late telophase of division of antheridium initial into primary spermatogenous cell and tube nucleus; this is the usual shedding stage of this species, with prothallials undivided.

FIG. 32.—Metaphase of division of antheridium initial showing the twelve gametophytic chromosomes; see also *fig. 27*.

FIG. 33.—Details of nucleus of primary spermatogenous cell of *P. totarra Hallii*.